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Original article

Influence of salinity stress on PSII in barley (*Hordeum vulgare* L.) genotypes, probed by chlorophyll-*a* fluorescence

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ABSTRACT

Objectives: Chlorophyll-*a* fluorescence is an efficient tool to determine the photosynthetic capacity of plants and the health status of plants under normal or stress conditions including salinity stress. This study was aimed to elucidate changes in the efficiency of photosystem II (PSII) in barley genotypes differing in degree of salt tolerance, which can be used for phenotyping in the breeding program for developing salt-tolerant cultivars.

Methodology: Twelve barley (*Hordeum vulgare* L.) genotypes were subjected to salt stress and salt stress reduced the growth of all barley genotypes, which is associated with a decline in chlorophyll and K^+ contents (roots and leaves) and increase in Na^+. Of the 12 barley genotypes, one salt-tolerant (B-10008) and one salt-sensitive barley genotype (B-14011) was selected to further investigate the structural stability of PSII using fast chlorophyll *a* kinetic analysis under salinity stress.

Results: The shape of OJIP transients changed due to salt stress in both salt-sensitive and salt-tolerant barley genotypes indicating a disturbance in structural stability at various points of PSII. The detailed analysis of JIP-test parameters suggested that salt stress caused photoinhibition of PSII due to enhanced inactive reaction centers, reduced absorption flux (ABS/RC), low transfer of electrons per reaction center (ET_O/RC) and enhanced accumulation of Q_{A^-} (V_J) thus reducing primary photochemistry (TR_O/RC, Φ_{PO}). These changes in PSII resulted in the reduction of the maximum quantum yield of PSII (Fv/Fm) and performance index (PI_{ABS}). Moreover, salinity stress enhanced dissipation energy flux per reaction center (DI_O/RC) as a protective measure to save PSII from photooxidative damage in thylakoid membrane.

Conclusion: The appearance of positive K and L-bands supported the idea that salt stress dissociated the light-harvesting complex from core proteins of PSII, damaged oxygen-evolving complex and reduced the structural stability of PSII by disturbing the electron transfer between acceptor and donor sides of PSII especially in salt sensitive genotype (B-14011). Moreover, such an adverse effect of salt stress on PSII was less in salt-tolerant barley genotype (B-10008). Thus, some JIP-test parameters can be used as potential phenotype marker for screening salt-tolerant genotypes.

1. Introduction

Photosynthesis is one of the most basic physiological and biochemical process of plant's growth and productivity, but it is severely affected by soil salinity (Isayenkov and Maathuis, 2019; Majeed and Muhammad, 2019). Salt stress causes the excessive uptake of Na^+ from root zone which creates osmotic and water stress to plants (Arif et al., 2020). Na^+ toxicity limits the uptake of other nutrients (K^+, Ca^{2+}, P, N) (Rehman et al., 2019), triggering

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disruption in biochemical, physiological and molecular activities of cell (Shahid et al., 2020). Salinity stress also reduces leaf water and osmotic potential with a concurrent increase in osmotic stress (Betzen et al., 2019; Cambridge et al., 2017) resulting into production of reactive oxygen species (ROS) like singlet oxygen ($^1O_2$), superoxide (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicles (OH^-) especially in the chloroplast and mitochondria (Ahammed et al., 2018; Li et al., 2019; Siddiqui et al., 2020; Yi et al., 2018). This salinity induced oxidative stress leads to the reduction in PSII activity by damaging reaction centers of photosystem II (PSII), oxygen evolving complex and reducing activity of quinine acceptor (Betzen et al., 2019; Kalaji et al., 2018).

Plant responses to salt stress depend on PSII activity and biochemical changes around PSII such as activation of xanthophyll cycle to dissipate excessive heat energy (Kalaji et al., 2018), accumulation of osmoprotectants such as glycinebetaine, proline to stabilize PSII (Zhang and Dai, 2019), and accumulation of antioxidants such as ascorbic acid as an alternative electron donor (Bose et al., 2017; Ogbaga et al., 2018). PSII, multi-subunit protein-pigment complex, is highly sensitive to salt stress (Messedi et al., 2016). Among protein complexes of PSII, D1 protein is a key target of salt-induced ROS thereby resulting in photoinhibition of PSII (Asrar et al., 2017). Moreover, salt-induced ROS also inhibits the repair cycle of D1 protein (Bose et al., 2017). Various studies suggested that salt-tolerant plants or salt-tolerant cultivars of the same species had greater PSII stability and activity which can be used as non-destructive phenotyping techniques in the breeding program (Bose et al., 2017; Iqbal et al., 2019). However, it is not yet clear that genotypes/cultivars differing in salinity tolerance use the common mechanism of salt tolerance.

The efficiency of PSII in plants can be detected by chlorophyll-*a* fluorescence (OJIP), a steadfast and non-destructive method, to monitor the physiological activity of photosynthetic machinery (Guidi et al., 2019). It also gives some valuable information about energy transfer during photosynthetic process (Kalaji et al., 2017). The name OJIP refers to the intermediate steps, starting with minimal fluorescence 'O' to maximum fluorescence 'P' with 'J' and 'I' are intermediate steps. The O-J part of the fluorescence curve represents primary electron acceptor (Q_A) reduction phase; J-I part represents the transport of electrons from Q_A (primary electron acceptor) to Q_B (secondary electron acceptor) and ultimately to plastocyanin (PC) through intermediate electron carriers plastoquinone (PQ) and cytochrome complex (Cyt b6f). Finally, the I-P part of the fluorescence curve represents the reduction of the final electron acceptor at PSI (Çiçek et al., 2017).

The 'JIP' test, based on chlorophyll-*a* fluorescence, is used to evaluate the response of photosynthetic apparatus to different abiotic stresses (Rastogi et al., 2020a). This test offers an enough data to understand the structure and function of PSII and the possible flow of electron in thylakoid membranes (Kalaji et al., 2011a). The 'JIP' test can be used to calculate some important parameters like energy fluxes absorbed (ABS/RC) and trapped (TRo/RC) per reaction center (RC), primary photochemistry (ψo), electron transport rate (ETo/RC), non-photochemical quenching for energy dissipation (DIo/RC), efficiency of donation electron to PSI (Fv/Fo), maximum quantum yield of PSII (Fv/Fm) and performance index on absorption basis (PI_{ABS}) (Küpper et al., 2019).

From the above-mentioned reports, PSII responses to salt stress in two barley genotypes differing in salt tolerance using fast chlorophyll *a* kinetic analysis were assessed. In this study, the first 12 barley genotypes were screened for salt tolerance using physiological attributes and subsequently OJIP analysis of two genotypes of barley was carried out.

2. Materials and methods

This study was conducted to evaluate the influence of salinity stress on PSII of barley (*Hordeum vulgare* L.) genotypes. The experiment was conducted at the Botanic Gardens, Bahauddin Zakariya University, Multan in complete randomize design (CDR). Twelve barley genotypes (B-5011, B-9006, B-9008, B-10008, B-14011, B-15005, H-93, Jou-83, Jou-87, 14003, 15,002 and 15003) were sown in plastic pots filled with thoroughly washed 10 kg sand. Ten days after complete germination salt stress (200 mM NaCl) was gradually applied to half of the plants as others remained as control. The plants were regularly irrigated with full strength Hoagland's Nutrient solution (Hoagland and Arnon, 1950) to fulfill nutritional requirements. The morphological attributes (biomass accumulation, shoot and root lengths), ionic contents (Na^+ and K^+) and photosynthetic pigments (chlorophyll-*a*, *b* and total) were examined in these barley genotypes for the screening of one salt-tolerant and one salt-sensitive genotype. While chlorophyll-*a* fluorescence attributes were studied only in selected barley genotypes (B-10008 and B-14011).

2.1. Chlorophyll contents

Photosynthetic pigments (chlorophyll-*a*, -*b* and total) were estimated following Arnon (1949) method. 0.1 g fresh leaf samples were grounded with 5 ml of 80%. Grounded material was centrifuged for 10,000 rpm for five minutes. The supernatant was used to measure the absorbance of samples at 645 nm and 663 nm using a spectrophotometer (U-2900).

2.2. Ionic (Na^+ and K^+) contents

The determination of Na^+ and K^+ contents were done in roots and leaves of each barley genotype. The oven-dried plant material (0.1 g) was digested indigestion mixture (Se, $LiSO_4{\cdot}2H_2O$ and H_2O_2) at 350 °C on hot plate. The diluted (50 ml) solution is used for the determination of Na^+ and K^+ contents in plant samples through flame photometer.

2.3. Chlorophyll-a fluorescence (OJIP)

Chlorophyll-*a* fluorescence (OJIP) transients were recorded from middle of fully expended leaf (2nd from top) after continuous maintenance of salt stress for eight days, using portable Fluor pen-100. Before taking readings, the leaves were covered with aluminum foils for 30 min to ensure closure of almost all reaction centers of PSII. As all the photosynthetic reaction centers were probably at ground state (closed), then supposedly, electron transport chain was also at relaxed state. It ensures the complete oxidation of Q_A, Q_B and PQ pool behind PSII reaction centers. The dark-adapted leaves were exposed to strong actinic light (3000 μmol photons/m^2s^1) and fluorescence was recorded form 20 μs to 2 s. The raw data of OJIP transient were plotted on log time scale. Structural and functional changes in PSII *via* various biophysical and phenomenological energy fluxes, of barley genotypes, under normal and saline conditions, were worked out from fluorescence data (Table 1).

The chlorophyll-*a* fluorescence transients were double normalized between F_O (20 μs) and F_K (300 μs) and were expressed as V_{OK} [$V_{OK} = \{(Ft - F_O) / (F_K - F_O)\}$] to understand the probability of fluorescence rise at K step (300 μs). While the kinetic differences between saline and non-saline transients of this phase were expressed as ΔV_{OK} or L-band (ΔV_{OK}). The transients were double normalized between F_O (20 μs) and F_J (2000 μs) and were

Table 1
Key parameters for chlorophyll-*a* fluorescence (OJIP) analysis emitted by dark adopted barley leaves.

Terms	Description	Reference
Fo	Minimum fluorescence at 20 μs (all RCs are supposed to be opened)	(Stirbet and Govindjee, 2011)
Fj	Fluorescence intensity at 2 ms at J phase of OJIP	(Strasser et al., 2000)
Fi	Fluorescence intensity at 30 ms at I phase of OJIP	
Fm	Maximum fluorescence intensity at P phase of OJIP (maximum RCs are supposed to be closed)	
Fv	Variable fluorescence (Fm-Fo)	
Fv/Fo	Efficiency of electron donation to PSII	(Mohammed et al., 1995)
Fv/Fm	Maximum quantum yield of PSII	(Strasser et al., 2000)
N	Maximum turn-overs of Q_A reduction until Fm was reached	
Mo	Maximum rate of accumulation of closed reaction centers	(Strasser et al., 2004)
ABS/RC	Absorption per reaction center at PSII / Ratio of active reaction centers in PSII	(Clark et al., 2000)
TRo/RC	Trapped energy flux per reaction center (t = 0)	(Force et al., 2003; Perboni et al., 2012)
ETo/RC	Electron transport flux per reaction center (t = 0)	
DIo/RC	Dissipation energy flux per reaction center (t = 0)	
ΦD_O	Quantum yield of energy dissipated	
ΦP_O	Maximum quantum yield of primary photochemistry (t = 0)	(Appenroth et al., 2001)
ΦEo	Quantum yield of electron transported to ETC beyond QA	(Pandey and Yeo, 2008)
Ψo	Probability that trapped electron was transferred to ETC beyond Q_A	(Appenroth et al., 2001)
PI_{AbS}	Performance index on absorption basis	(Appenroth et al., 2001; Mathur et al., 2011)

expressed as V_{OJ} [$V_{OJ} = \{(Ft - F_O) / (F_J - F_O)\}$] and the kinetic difference between saline and non-saline transients were expressed as K-band (ΔV_{OJ}). Moreover, the fluorescence transients were double normalized between 'O' (F_O) and 'P' (F_P) and difference between double normalized saline and non-saline barley plants were calculated and expressed as V_{OP} [$V_{OP} = (Ft - F_O)/(F_P - F_O)$]. Furthermore, to understand the changes in O-I phase fluorescence data was double normalized between F_O (20 μs) and F_I (<1 s) and were expressed as V_{OI} [$(V_{OI} = (Ft - F_O)/(F_I - F_O)$], while the changes in I-P phase were determined by double normalizing the fluorescence data between F_I (20 μs) and F_P (200 ms) expressed as V_{IP} [$(V_{IP} = (Ft - F_O)/(F_P - F_O)$].

3. Statistical analysis

The recorded data of twelve barley genotypes grown at two salinity levels was subjected to two-way analysis of variance (ANOVA) using SPSS-16.0 statistical package (SPSS Inc. Chicago, IL, USA). Statistical significance difference at 5% level ($P < 0.05$) among barley genotypes was estimated by Duncan's Multiple Range Test (DMRT).

4. Results

4.1. Plant growth

Analysis of variance (ANOVA) of the data regarding different morphological attributes (fresh and dry weights, shoot and root lengths) displayed a highly significant ($p < 0.001$) reduction at 200 mM NaCl salinity stress. Plant fresh weights and dry weights were reduced remarkably from 20% to 77.8% and from 18.8% to 75.8% respectively under salt stress. Similarly, shoot lengths and root lengths were decreased from 20.4% to 66.5% and from 10.1% to 44.5% respectively Overall genotype "B-10008" showed better accumulation of biomass and plant height while "B-14011" was the lowest in maintaining growth with respect to all other genotypes (Table 2).

4.2. Photosynthetic pigments (Chlorophyll contents)

Photosynthetic pigments (chlorophyll-*a*, -*b* and total) were highly significantly ($p < 0.001$) affected at 200 mM NaCl stress. The imposition of NaCl to growing media caused a reduction in Chlorophyll-*a* contents from 14% to 41%, chlorophyll-*b* contents were reduced from 12%, to 46.8% and similarly, total chlorophyll contents were decreased from 10.3% to 50.3% respectively. Contrary to the reduction in chlorophyll-*b* contents were enhanced to 19.4% in H-93 and 13.2% in Jou-83 (Table 3).

4.3. Root and leaf ionic contents (Na^+ and K^+)

The root and leaf K^+ contents were prominently decreased due at 200 mM NaCl. Lowest decrease (47%) in root and leaf (23%) K^+ contents were observed in genotype (B-10008) at 200 mM NaCl while at this salinity level 75% decrease in root K^+ contents was observed in "H-93" and 67% decrease was observed in "B-14011" (Fig. 1). The imposition of salinity in growing media dramatically enhanced Na^+ contents and decreased K^+ contents in roots and leaves. Root Na^+ contents were enhanced from 265% (B-9008) to 969% (H-93) while the accumulation of Na^+ in leaves were enhanced form 444% (B-10008) to 1083% (B-14011) (Fig. 2).

4.4. Fast fluorescence kinetics (OJIP)

Both barley genotypes produced a typical polyphasic fluorescence induction transients (Fig. 3). Minimal fluorescence (F_O) reflected that Q_A were in the oxidized state (Open PSII reaction centers) and Fm showed that Q_A were in the reduced state (Close PSII reaction centers). Results of raw OJIP transients showed that salt stress decreased the fluorescence mainly at I and P steps in both barley genotypes and differential kinetics verified this (Fig. 3). Moreover, the extent of this decreased became more evident in salt-sensitive genotype (B-14011) at 'I' and 'P' steps. Both genotypes showed an equal response in O-J (primary photochemistry) regions but both genotypes differed at J-I and I-P (redox states of Q_A, PQ and acceptor site of PSI) region (Fig. 3). The value of V_J was significantly high in "B-14011" which indicated that closed reaction centers were accumulating under salinity stress in this genotype. Salt stress caused the appearance of both K and L-bands which were more obvious in genotype "B-14011" showing that salt stress damaged OEC and its connection with LHC of core PSII proteins on donor end (Fig. 5).

4.5. Performance of PSII (JIP test)

Salt stress reduced the quantum yield and efficiency of PSII as measured ratios of basic chlorophyll fluorescence parameters in both barley genotypes (Fig. 4). The quantum yield of primary photochemistry (ΦPo), the efficiency of PSII to move trapped electrons in electron transport chain (Ψo) and electron transport to beyond Q_A (ΦE_O), were substantially reduced due to salinity stress, particularly in "B-14011" (salt-sensitive genotype) as compared to "B-10008" (salt-tolerant genotype) (Fig. 4). Moreover, salt stress increased the quantum yield of heat dissipation (ΦD_O) in both bar-

Table 2
Mean (±S.E) of plant fresh weights (g), plant dry weights (g), shoot lengths (cm) and root lengths (cm) of twelve barley genotypes grown under control (0 mM NaCl) and saline (200 mM NaCl) conditions.

Genotypes	Plant fresh weights (g)		Plant dry weights (g)		Shoot lengths (cm)		Root lengths (cm)	
	Control	200 mM NaCl	Control	200 mM NaCl	Control	200 mM NaCl	Control	200 mM NaCl
B-5011	8.89 ± 0.48 ^{cd}	2.39 ± 0.39 ^{g}	0.89 ± 0.05 ^{cd}	0.27 ± 0.05^{e}	33.33 ± 1.83^{bcd}	13.37 ± 1.23 ^{cd}	9.60 ± 0.72^{de}	5.17 ± 0.19^{de}
B-9006	12.90 ± 0.20^{b}	9.38 ± 0.32^{bc}	1.33 ± 0.04^{b}	0.99 ± 0.07^{b}	48.43 ± 0.92^{a}	37.00 ± 4.54^{a}	12.85 ± 0.86^{b}	10.13 ± 0.63^{b}
B-9008	8.74 ± 0.39 ^{cd}	3.46 ± 0.17^{f}	0.88 ± 0.04 ^{cd}	0.42 ± 0.08^{d}	27.37 ± 1.67^{ef}	13.13 ± 1.39 ^{cd}	10.13 ± 0.09 ^{cd}	8.10 ± 0.64^{c}
B-10008	14.86 ± 0.43^{a}	11.85 ± 0.35^{a}	1.49 ± 0.02^{a}	1.27 ± 0.05^{a}	48.93 ± 0.73^{a}	38.93 ± 2.10^{a}	14.83 ± 0.22^{a}	12.10 ± 0.49^{a}
B-14011	6.18 ± 0.40^{e}	1.37 ± 0.12 ^{h}	0.61 ± 0.04^{e}	0.15 ± 0.01^{e}	25.55 ± 1.56^{f}	8.57 ± 0.81^{d}	6.10 ± 0.40 ^{g}	2.60 ± 0.40^{f}
B-15005	8.72 ± 0.32 ^{cd}	5.71 ± 0.26^{d}	0.86 ± 0.04 ^{cd}	0.67 ± 0.03^{c}	35.07 ± 1.45^{bcd}	18.93 ± 0.82^{bc}	9.77 ± 0.27^{de}	6.30 ± 0.32^{d}
H-93	9.69 ± 0.33^{c}	4.72 ± 0.26^{e}	0.96 ± 0.03^{c}	0.47 ± 0.03^{d}	37.60 ± 1.17^{b}	22.70 ± 1.13^{b}	11.33 ± 0.33^{bc}	9.00 ± 0.70^{bc}
Jou-83	12.72 ± 0.76^{b}	10.15 ± 0.10^{b}	1.33 ± 0.05^{b}	1.02 ± 0.04^{b}	51.67 ± 0.78^{a}	39.33 ± 2.19^{a}	12.25 ± 0.58^{b}	9.33 ± 0.61^{bc}
Jou-87	8.02 ± 0.44^{d}	2.7 ± 0.26 ^{fg}	0.81 ± 0.05^{d}	0.28 ± 0.02^{e}	32.53 ± 1.55 ^{cd}	12.73 ± 1.18 ^{cd}	9.02 ± 0.74^{def}	5.00 ± 0.32^{de}
14,003	12.13 ± 0.12^{b}	8.62 ± 0.35^{c}	1.28 ± 0.03^{b}	1.04 ± 0.05^{b}	47.40 ± 1.13^{a}	35.37 ± 1.73^{a}	12.17 ± 0.50^{b}	9.37 ± 0.41^{bc}
15,002	7.87 ± 0.31^{d}	2.54 ± 0.50 ^{fg}	0.80 ± 0.02^{d}	0.26 ± 0.05^{e}	31.43 ± 2.07^{de}	11.38 ± 1.57^{d}	7.63 ± 0.13^{f}	3.93 ± 0.23^{ef}
15,003	9.69 ± 0.44^{c}	4.50 ± 0.40^{e}	0.99 ± 0.07^{c}	0.46 ± 0.04^{d}	36.50 ± 2.10^{bc}	13.00 ± 1.45 ^{cd}	8.37 ± 0.19^{ef}	4.00 ± 0.12^{ef}
SOV	**Df**							
Genotypes (G)	11	54.24***	0.617***		652.97***		43.79***	
Salt (S)	1	315.08***	3.02***		4477.65***		190.35***	
G × S	11	2.28***	0.031**		26.47**		0.918 ^{ns}	
Error	48	0.403	0.006		0.698		0.676	

Values are means of salt treatments (0 and 200 mM) with n = 3 replicates of each treatment.
Different letters represent statistical significance difference estimated by Duncan's Multiple Range Test (DMRT) among barley genotypes (P < 0.05).
***= Significance level at 0.001, **= Significance level at 0.01, ns = non-Significant.

Table 3
Mean (±S.E) of chlorophyll-*a*, chlorophyll-*b* and total chlorophyll (mg 10ml^{1-}f.wt.) contents of twelve barley genotypes grown under control (0 mM NaCl) and saline (200 mM NaCl) conditions.

Genotypes	Chlorophyll-*a* (mg 10ml^{1-} f.wt.)		Chlorophyll-*b* (mg 10ml^{1-} f.wt.)		Total chlorophyll (mg 10ml^{1-} f.wt.)	
	Control	200 mM NaCl	Control	200 mM NaCl	Control	200 mM NaCl
B-5011	0.0128 ± 0.0002^{b}	0.0104 ± 0.0002 ^{cd}	0.0045 ± 0.0002^{ab}	0.0034 ± 0.0001^{bc}	0.0156 ± 0.0011^{de}	0.0117 ± 0.0009^{ef}
B-9006	0.0125 ± 0.0001^{b}	0.0106 ± 0.0005 ^{cd}	0.0050 ± 0.0001^{ab}	0.0044 ± 0.0004^{a}	0.0186 ± 0.0005^{abc}	0.0154 ± 0.0002^{abc}
B-9008	0.0148 ± 0.0004^{a}	0.0110 ± 0.0003^{c}	0.0051 ± 0.0001^{a}	0.0038 ± 0.0002^{ab}	0.0179 ± 0.0004^{bc}	0.0140 ± 0.0005 ^{cd}
B-10008	0.0153 ± 0.0005^{a}	0.0131 ± 0.0001^{a}	0.0050 ± 0.0001^{ab}	0.0041 ± 0.0002^{ab}	0.0196 ± 0.0004^{a}	0.0160 ± 0.0005^{ab}
B-14011	0.0101 ± 0.0006^{c}	0.0059 ± 0.0001 ^{h}	0.0038 ± 0.0003^{c}	0.0023 ± 0.0001^{d}	0.0132 ± 0.0004^{f}	0.0066 ± 0.0004 ^{g}
B-15005	0.0130 ± 0.0001^{b}	0.0097 ± 0.0001^{d}	0.0046 ± 0.0002^{ab}	0.0027 ± 0.0001 ^{cd}	0.0170 ± 0.0008 ^{cd}	0.0114 ± 0.0008^{ef}
H-93	0.0106 ± 0.0001^{c}	0.0075 ± 0.0004 ^{fg}	0.0029 ± 0.0001^{d}	0.0035 ± 0.0001^{b}	0.0154 ± 0.0005^{e}	0.0138 ± 0.0009 ^{cd}
Jou-83	0.0153 ± 0.0003^{a}	0.0129 ± 0.0002^{ab}	0.0035 ± 0.0003^{c}	0.0040 ± 0.0001^{ab}	0.0188 ± 0.0001^{ab}	0.0168 ± 0.0005^{a}
Jou-87	0.0134 ± 0.0005^{b}	0.0083 ± 0.0005^{ef}	0.0045 ± 0.0002^{ab}	0.0033 ± 0.0001^{bc}	0.0174 ± 0.0006^{bc}	0.0129 ± 0.0007^{de}
14,003	0.0146 ± 0.0002^{a}	0.0120 ± 0.0003^{b}	0.0047 ± 0.0002^{ab}	0.0039 ± 0.0001^{ab}	0.0198 ± 0.0002^{a}	0.0143 ± 0.0003^{bcd}
15,002	0.0103 ± 0.0004^{c}	0.0071 ± 0.0003 ^{g}	0.0044 ± 0.0002^{b}	0.0023 ± 0.0003^{d}	0.0140 ± 0.0003^{ef}	0.0075 ± 0.0003 ^{g}
15,003	0.0109 ± 0.0005^{c}	0.0087 ± 0.0002^{e}	0.0035 ± 0.0002^{c}	0.0025 ± 0.0004^{d}	0.0143 ± 0.0002^{ef}	0.0106 ± 0.0006^{f}
SOV	**df**					
Genotypes (G)	11	2.62×10^{-5}***	2.04×10^{-6}***		4.19×10^{-5}***	
Salt (S)	1	1.45×10^{-4}***	1.58×10^{-5}***		3.18×10^{-4}***	
G × S	11	1.44×10^{-6}**	9.55×10^{-7}***		2.890×10^{-6}***	
Error	48	3.34×10^{-7}	1.29×10^{-7}		9.26×10^{-7}	

Values are means of salt treatments (0 and 200 mM) with n = 3 replicates of each treatment.
Different letters represent statistical significance difference estimated by Duncan's Multiple Range Test (DMRT) among barley genotypes (P < 0.05).
***= Significance level at 0.001, **= Significance level at 0.01.

ley genotypes. Data analysis shows that the maximum number of turns over for Q_A reduction until Fm reached (N), rate of Q_A reduction (Mo) and maximum turn-over of Q_A reduction until Fm reached (N) were reduced.

Results revealed that salinity stress caused a significant reduction in energy flux for absorption (ABS/RC), trapping (TRo/RC) and electron transport (ETo/RC) in both genotypes of barley. In contrast, dissipation energy flux per reaction center (DIo/RC) was significantly enhanced in both genotypes at 200 mM NaCl stress. Efficiency of electron donation to PSI (Fv/Fo) and maximum quantum yield of PSII (Fv/Fm) was significantly reduced under salinity stress in both genotypes. The area between 'Fo' and 'Fm' was also decreased under salinity stress which indicated that inhibition in transport of electron from RC to PQ pool. Similarly, performance index on the absorption basis (PI_{ABS}) was also reduced under salinity stress (Fig. 4). The adverse effects of salt stress on these attributes were greater in salt-sensitive genotype "B-14011" as compared to salt tolerant genotype "B-10008" (Fig. 4).

The K and L-bands were appeared due to imposition of salinity stress in both barley genotypes. A clear difference in these bands was observed in both genotypes (Fig. 5). Similarly, a remarkable difference was observed in salt sensitive genotype (B-14011) at V_{OI} and V_{IP} stage of fluorescent curves (Fig. 6).

5. Discussion

Soil salinity is a major global issue to sustainable agriculture as it affects plant growth and development at all growth stages by disturbing cellular and physiological processes (Arif et al., 2020). Salinity causes osmotic stress which in turn leads the plant to physiological drought condition thus results in stunted plant growth (Ahammed et al., 2020). In this study, salt stress reduced the growth (biomass accumulation, shoot and root lengths) of all 12 barley genotypes; however, this adverse effect was different on different genotypes. Based on growth under salt stress, one

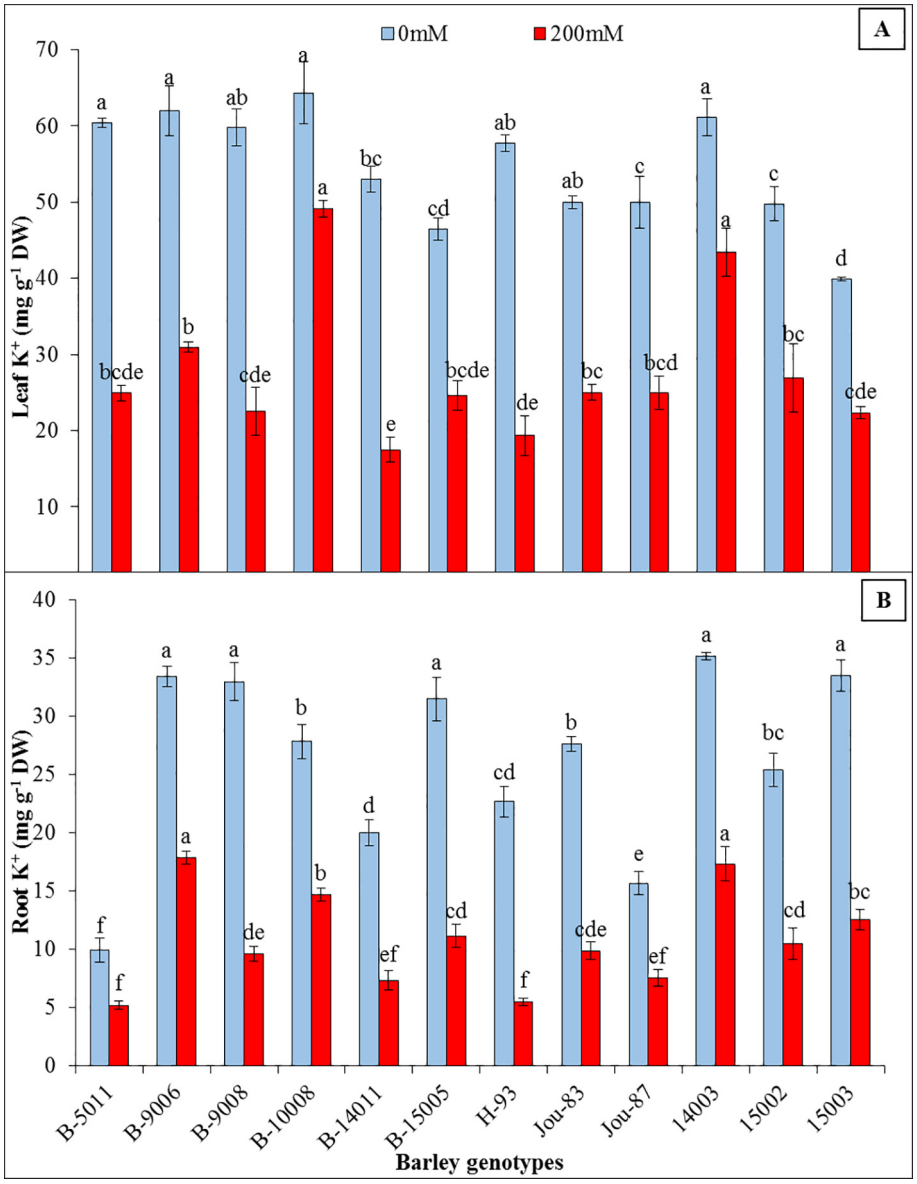

Fig. 1. Leaf and root K^+ contents (mg g^{-1} DW) of twelve barley genotypes grown under control (0 mM NaCl) and saline (200 mM NaCl) conditions. Different letters represent statistical significance difference estimated by Duncan's Multiple Range Test (DMRT) among barley genotypes ($P < 0.05$) at control (0 mM NaCl) and saline (200 mM NaCl) conditions independently.

salt-tolerant (B-10008) and one salt-sensitive genotype (B-14011) were identified (Tables 2 and 3). This reduction in growth attributes is due to excessive accumulation of Na^+ and Cl^- as well as unbalanced nutrient uptake as reported in barley plants (Shelden et al., 2020). Similar genotypic variation for salt tolerance has already been observed in barley (Saade et al., 2020), rice (Subudhi et al., 2020), canola (Iqbal et al., 2019; Ulfat et al., 2020). Such genotypic variation for salt tolerance could be due to variation in different physiological processes such as photosynthetic capacity, ion uptake and maintenance of plant water status or antioxidant potential (Subudhi et al., 2020).

Salinity stress is responsible for excessive accumulation of Na^+ with simultaneous reduction in the uptake of essential nutrients like K^+ from roots to photosynthetic leaves (Zhang et al., 2020). It has also been reported that the accumulation of Na^+ and a decrease in K^+ contents in photosynthetic leaves may result in chlorophyll degradation and disturbs thylakoid membranes (Bose et al., 2017). In this study, a greater increase in the accumulation of Na^+ and a decrease in K^+ in leaves and roots of all barley genotypes was observed, however this increase was much high of salt-sensitive genotype (B-14011) as compared to its control plants (Figs. 1 and 2). Similar results were observed in wheat (Iqra et al., 2020), canola (Naveed et al., 2020), maize (Azizian and Sepaskhah, 2020), tomato (Kamanga et al., 2020), rice (Qin and Huang, 2020) and barley (Zeeshan et al., 2020). Several studies found that accumulation of Na^+ and K^+ both change the stacking of grana in the chloroplast and thus light-driven reactions (Bose et al., 2017).

Photosynthetic pigments are an important determinant of plant photosynthetic capacity (Khan et al., 2019). Chlorophyll *a* and chlorophyll *b* are integral component of PSII. Salt stress reduced these photosynthetic pigments (Table 3), which might have been due to either decrease in biosynthesis or increase in degradation of these pigments by stress-induced activation of chlorophyllase activity (Mihailovic et al., 1997). Salinity reduced the total chlorophyll contents in wheat (Betzen et al., 2019), tomato (Kamanga

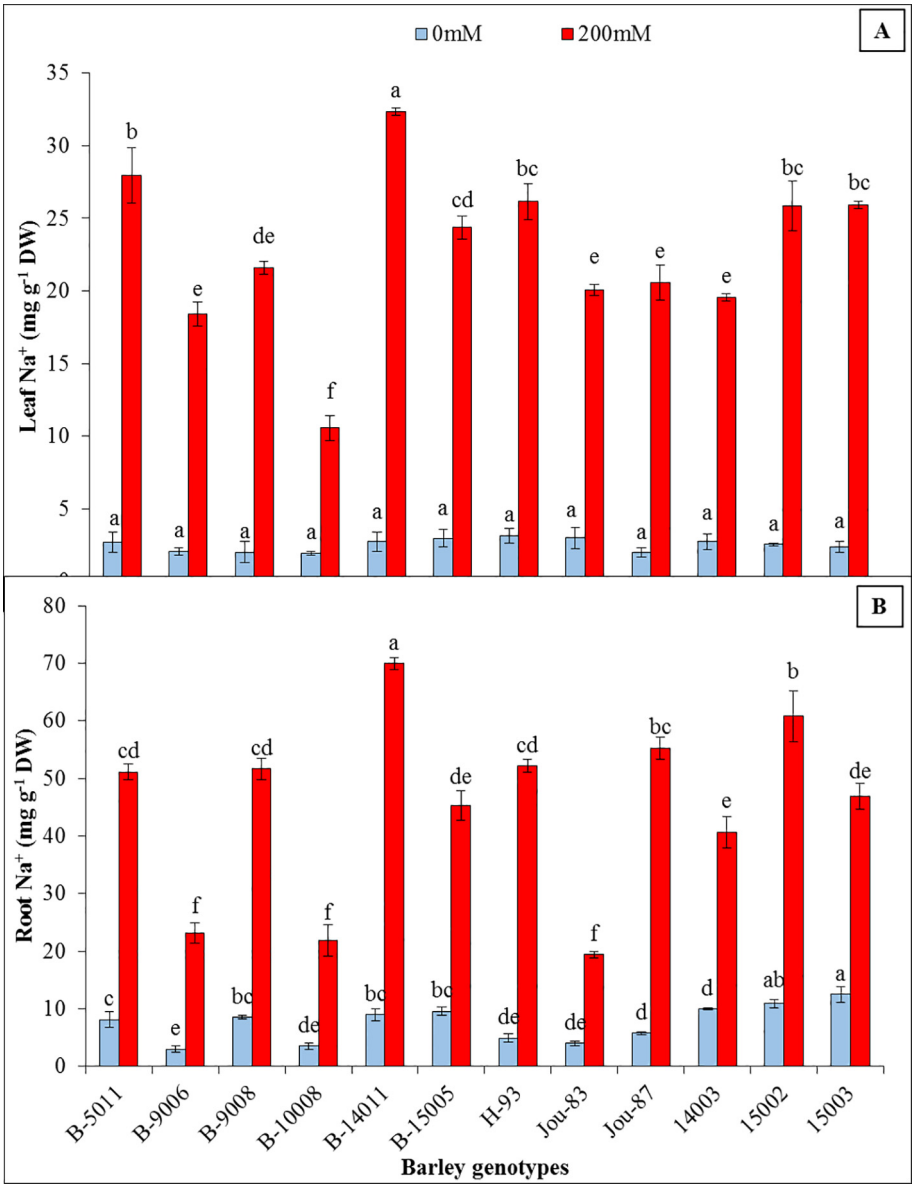

Fig. 2. Leaf and root Na^+ contents (mg g^{-1} DW) of twelve barley genotypes grown under control (0 mM NaCl) and saline (200 mM NaCl) conditions. Different letters represent statistical significance difference estimated by Duncan's Multiple Range Test (DMRT) among barley genotypes ($P < 0.05$) at control (0 mM NaCl) and saline (200 mM NaCl) conditions independently.

et al., 2020), wheat and barley (Zeeshan et al., 2020). There is a direct relationship of chlorophyll contents and electron transport chain in photosynthesis (Ahammed et al., 2018).

OJIP analysis is the most powerful and widely used technique to understand the structural stability of PSII as it gives a complete insight of energy fluxes between different components of PSII (Guidi et al., 2019). In this experiment, we have observed that salt stress reduced the fluorescence emission in both barley genotypes, particularly in salt-sensitive genotype (B-14011), at all steps of OJIP transient (O, J, I and P steps i.e., F_O, F_J, F_I and Fm), however, this decrease was much great at I and P steps (Figs. 3 and 4). The genotype "B-14011" exhibited very low 'Fm' under salinity stress. This low Fm value indicates the accumulation of inactive RC at PSII (Kalaji et al., 2011a). These results indicated that salt stress limited the transfer of absorbed energy from the light-harvesting complex to the reaction center and the probability of electron transport from donor end of PSII to acceptor side of PSII (Stirbet and Govindjee, 2011; Tsimilli-Michael and Strasser, 2008). The I-P phase of the curve was more strongly disturbed in salt-sensitive barley genotype "B-14011" which indicated poor redox state of pool of Q_B, *cyt*b_6f and acceptor end of PSI (Stirbet and Govindjee, 2011). Kinetically, response of I-P in fluorescence transient is correlated with PSI activity, so any change in I-P amplitude can be used as an indicators for change in PSI content of the leaf (Ceppi et al., 2012). The decrease in I-P phase reveal to enhanced cyclic electron flow (CEF) around PSI (Kono et al., 2014; Zhou et al., 2019) due to blockage of electron transfer at electron acceptor side of PSI (Hamdani et al., 2015).

Differential qualitative analysis of K-band and L-band (0–300 and 0–2000 s^{-1}) indicate the intactness of OEC and LHC with core PSII proteins or level of injuries to PSII at OEC and LHC sites (Rastogi et al., 2020a). Although salinity stress caused the appearance of K and L-bands in both genotypes still the intensity of damage was more obvious in genotype "B-14011" as compared to "B-10008" (Figs. 5 and 6). This showed that salinity induced damage to PSII at the donor end of PSII and LHC sites in genotype "B-

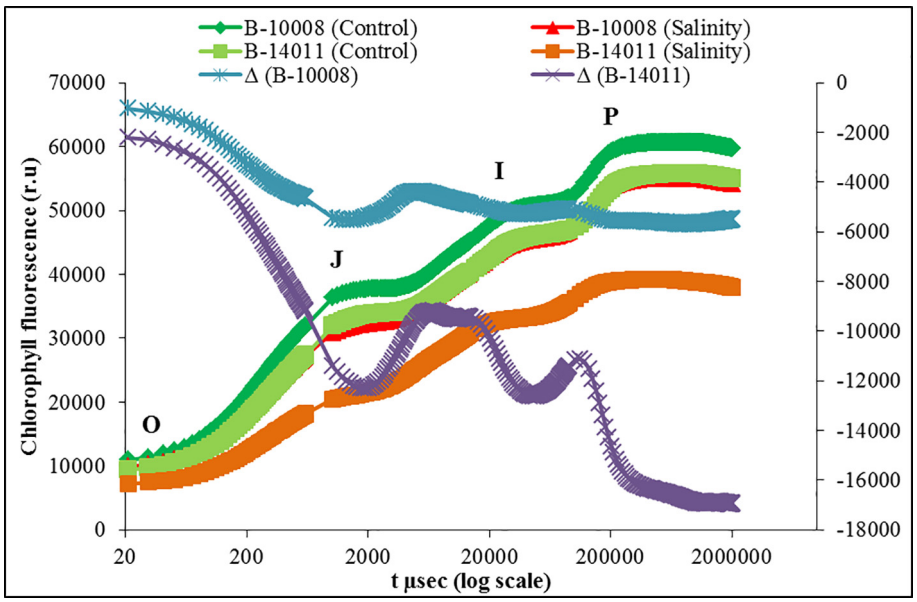

Fig. 3. Raw OJIP chlorophyll-*a* transients and kinetic differences of two barley genotypes (B-10008 and B-14011) grown under control (0 mM NaCl) and saline (200 mM NaCl) conditions.

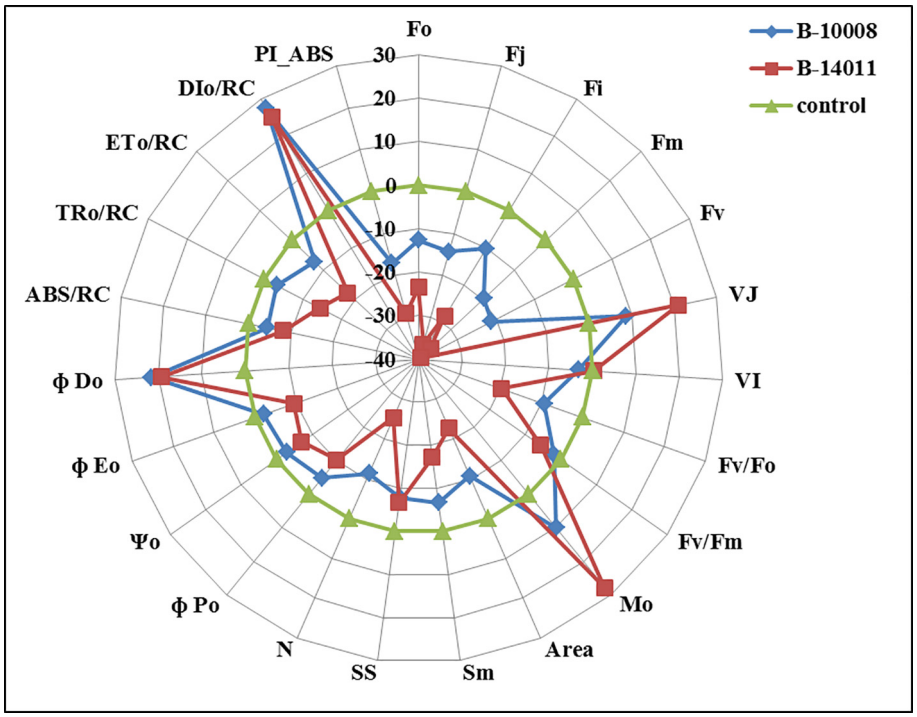

Fig. 4. A spider plot of selected JIP parameters derived from chlorophyll-*a* fluorescence in barley genotypes "B-10008" and B-14011" grown under non-saline (0 mM NaCl) and saline (200 mM NaCl) conditions.

14001" was more apparent due to presence of positive K and L-band.

Salinity stress more significantly reduced Fv/Fo and Fv/Fm in "B-14011" as compared to "B-10008" (Fig. 4). The Fv/Fo is the most sensitive component of photosynthetic electron transport chain (Fricke and Peters, 2002). The reduction in Fv/Fo indicates that efficiency of electron donation from OEC to donor side of PSII was reduced under salinity stress (Pereira et al., 2000). The decrease in Fv/Fm indicated the fact that PSII RCs were damaged/photochemically inactive under salinity stress. This may also be attributed to reduced capacity of PSII to transport electrons under salinity stress (Basu et al., 1998). These results are similar to some earlier studies in mustard (Wani et al., 2019) canola (Athar et al., 2015; Iqbal et al., 2019; Ulfat et al., 2020) and barley (Kalaji et al., 2011b). Similarly, significant enhancement in 'V_J' and 'Mo' under salt stress especially in salt sensitive genotype (B-14011) reflects the accumulation of $Q_{\bar{A}}$, due to blockage of electron transfer from Q_A to Q_B on the PSII acceptor side (Mehta et al., 2010).

Salt induced reduction in ABS/RC in both barley genotypes (Fig. 4) indicated that salt stress one hand resulted into increase

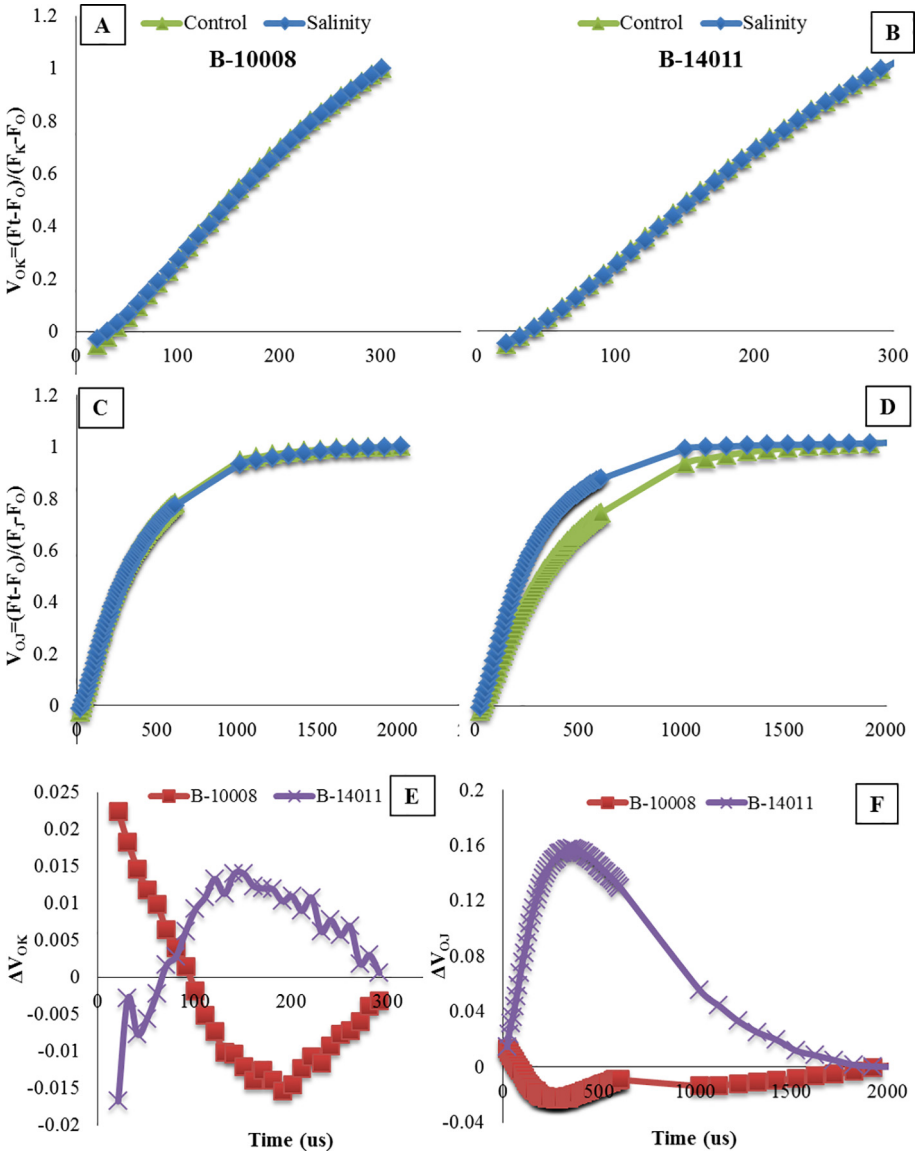

Fig. 5. Double normalized transients of chlorophyll-*a* fluorescence of dark adopted leaves of barley genotypes (B-10008 and B-14011) grown under non-saline and saline (200 mM NaCl) conditions. (A, B) Kinetic difference of V_{OK} [$(\Delta V_{OK} = (F_t\text{-}F_o)/(F_K\text{-}F_O)$] presenting L-band (E). (C, D) Kinetic difference of V_{OJ} [$(\Delta V_{OJ} = (Ft\text{-}F_O)/(F_J\text{-}F_O)$] presenting K-band (F).

in inactive RCs while on the other hand weakened the connectivity of PSII reaction center and antenna (LHC) (Kalaji et al., 2011a). The increase in inactive reaction centers due to salt stress results in the down-regulation of ETo/RC and ψ_O in both barley genotypes, however the tolerant genotype "B-10008" exhibited higher value for ETo/RC and ψ_O under salinity stress (Fig. 4). Similar results were observed previously in Syrian barley and sweet sorghum (Kalaji et al., 2011b; Rastogi et al., 2020a). The accumulation of inactive reaction centers during salinity stress, as was observed in this experiment, especially in salt sensitive genotype "B-14011", was associated with higher dissipation of absorbed energy as indicated by higher values of ΦD_O and DI_O/RC as an adaptive strategy to reduce photodamage to photosynthetic apparatus (Rastogi et al., 2020b).

Similarly, the quantum efficiencies of ΦPo and ΦEo were also decreased greater in salt-sensitive genotype (B-14011) (Fig. 4). These findings are similar to previous findings (Duarte et al., 2017; Kalaji et al., 2017; Küpper et al., 2019) which revealed that salt stress reduced the utilization of trapped photon in the transfer of electrons from Q_A to Q_B and beyond than Q_B to electron transport chain. Thus causing over-reduction of plastoquinone pool (PQH_2) under salinity stress (Rastogi et al., 2020a). Our results are also in conformity that salt stress caused a greater decrease in performance index on absorption basis (PI_{ABS}) which is linked with a decrease in active reaction centers, trapping and transport of electrons to ETC (Kalaji et al., 2011a; Rastogi et al., 2020a).

6. Conclusions

The JIP test gives a better understanding of the structure and function of PSII in barley genotypes under salinity stress. Salt stress reduced primary photochemistry of PSII by reducing the absorption of solar energy or size of antenna (ABS/RC), transfer of absorbed energy from the antenna to reaction center (TRo/RC) and utilization of trapped energy in electron transport (ET_O/RC). It is suggested that salt stress disrupted the light-harvesting complex from the reaction center and slightly affected the donor end of PSII. Such adverse effects of salt stress were greater in salt-

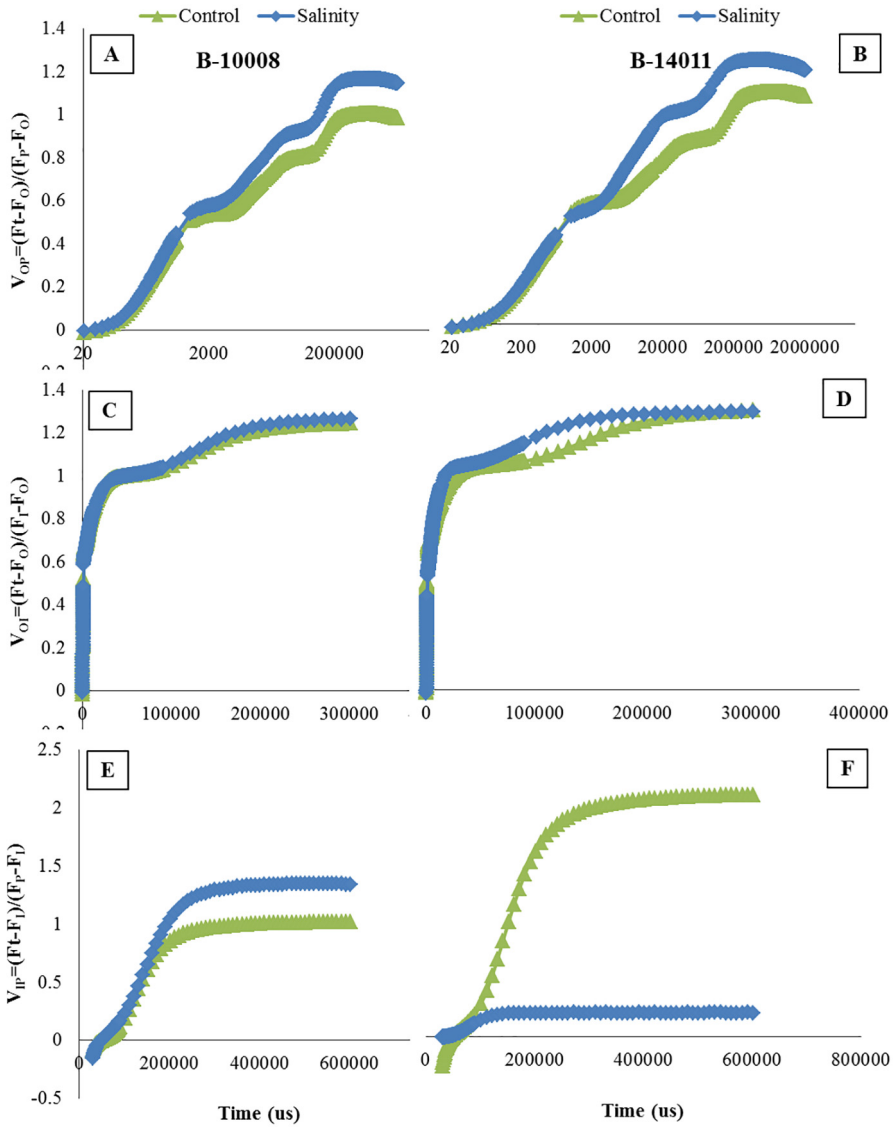

Fig. 6. Double normalized transients of chlorophyll-*a* fluorescence of dark adopted leaves of barley genotypes (B-10008 and B-14011) grown under non-saline and saline (200 mM NaCl) conditions. Kinetic difference of V_{OP} [$(\Delta V_{OP} = (F_t\text{-}F_O)/(F_P\text{-}F_O)$] (A, B), Kinetic difference of V_{OI} [$(\Delta V_{OI} = (Ft\text{-}F_O)/(F_I\text{-}F_O)$] (C, D) and Kinetic difference of V_{IP} [$(\Delta V_{IP} = (Ft\text{-}F_O)/(F_M\text{-}F_I)$] (E, F).

sensitive genotype (B-14011) as compared to tolerant one (B-10008).

CRediT authorship contribution statement

Muhammad Salim Akhter: Conceptualization, Methodology, Investigation. **Sibgha Noreen:** Conceptualization, Validation, Resources, Visualization, Supervision, Project administration. **Seema Mahmood:** Methodology. **Habib-ur-Rehman Athar:** Validation, Formal analysis, Data curation. **Muhammad Ashraf:** . **Abdulaziz Abdullah Alsahli:** Software, Formal analysis, Funding acquisition. **Parvaiz Ahmad:** Validation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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